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Phialophora sessilis, a species causing flyspeck signs on bamboo in China

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Abstract—*Phialophora sessilis* is reported and redescribed from China. It is distinguished from the other known species in the genus by reduced, flaring phialidic collarettes and clusters of single-celled conidia. ITS sequence analysis of four strains from Xianning, Hubei, China, attributed to the species shows it to be clearly distinct.

Key words—phialide, taxonomy, genetic analysis, sooty blotch, *Gramineae*

Introduction

The genus *Phialophora,* which was introduced by Medlar for *P. verrucosa* Medlar isolated from a human skin lesion (Medlar 1915), is currently regarded as a member of *Herpotrichiellaceae* (Haase et al. 1999). It is a little-differentiated genus of more or less pigmented, phialidic hyphomycetes (Hoog et al. 2000). With the addition of numerous species, the genus has become grossly polyphyletic, although some taxa have already been segregated from *Phialophora* into *Cadophora* (*Helotiales*), *Harpophora* (*Magnaporthaceae*), *Lecythophora* (*Coniochaetaceae*) and *Phaeoacremonium* (*Togniniaceae*) (Kirk et al. 2008).

Most *Phialophora* species are common saprobes in soil, wood pulp, and other plant material. Others are more specialized plant pathogens, and human pathogenicity is known for a few species (Gams 2000). *Phialophora sessilis* was first reported by Hoog et al. (1999) from *Picea abies* resin in the Netherlands

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and described in a comparative study of 34 strains belonging to the *Phialophora verrucosa* complex. Additional strains of *Ph. sessilis* originated from forest soils in Sweden, the lichen *Peltigera polydactyla* (Hoog et al. 1999), and marble powder in Italy (Caretta et al. 2006). Important phenetic characteristics of *Ph. sessilis* are dark, slow growing colonies, conspicuous collarettes that are darker than the rest of the phialide and inserted laterally on undifferentiated hyphae, and conidia sometimes inflating and then frequently bearing phialidic collarettes (Caretta et al. 2006).

During a recent survey of host plants for flyspeck fungi in China, two bamboo species were found to be hosts of flyspeck related with *Phialophora sessilis*.

Materials and methods

Fungal strains

Four strains were isolated from the culms of two different hosts; isolates ZJ81-D5 and ZJ81-D7 were from *Phyllostachys meyeri*, and ZJ88-B3 and ZJ88-B8 were from *Yushania falcatiaurita*. Representative dried culture and plant specimens were deposited in the Fungal Herbarium of Northwest A&F University (HMUABO), Yangling, Shaanxi Province, China.

Isolates

Individual sclerotium-like bodies (Batzer et al. 2005), growing in clusters on bamboo culms, were transferred to slants containing potato dextrose agar (200 g peeled potato, 20 g dextrose, 10 g agar in 1 L water; PDA) and cultured at 22 ± 1°C in the dark (Sun et al. 2003). Axenic cultures from slants were transferred to new PDA plates, a sterile cover slip was partially inserted into the agar adjacent to the colony and angled away from the colony at approximately 60 degrees to the agar surface in order to enable the fungus to grow onto the cover slip. Measurements of fungal structures were conducted based on isolates growing on cover slips. Colony descriptions were made after 1 month of growth on PDA plates at 22 ± 1°C in darkness.

DNA extraction, PCR, and sequencing

The protocol of Barnes et al. (2001) was followed to extract genomic DNA from fungal mycelium growing on PDA slants. The primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify part of the nuclear ribosomal RNA (nrRNA) operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the 5' end of the 28S rRNA gene. The PCR reaction mixture, consisting of 1 unit Taq polymerase, 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM of each primer, and 2 µL template DNA, was made up to a total volume of 25 µL with sterile water. Reactions were performed on a Bio-Rad PCR System PTC-200TM and the cycling conditions were an initial denaturation at 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 30 seconds, and a final 10-minute extension step at 72°C. Purifying and automated sequencing with the

primer ITS4/ ITS1-F of the PCR product was performed at Organism Technology Co., Shanghai, China.

Sequence alignment and phylogenetic analyses

The ITS nucleotide sequences generated in this study were added to sequences of six species of *Phialophora* obtained from GenBank (www.ncbi.nlm.nih.gov) (Table 1). After importing into BioEdit 5.0.9.1 (Hall 1999), all sequences were pruned to include the complete sequences of ITS1, the 5.8S rDNA gene, and ITS2 to aid alignment. Preliminary alignments of the ITS sequences were conducted using CLUSTAL-X (Thompson et al. 1997), with manual adjustment using BioEdit for visual improvement where necessary.

Table 1. Sequences used in the phylogenetic analysis

Species	GenBank	Reference
Cadophora gregata	AY249070	Harrington & McNew 2003
	AY249071	Harrington & McNew 2003
	U66727	Chen et al. 1996
	U66728	Chen et al. 1996
	U66729	Chen et al. 1996
Cadophora malorum	DQ317328	Arenz et al. 2006
	AF083201	McKemy et al. 2005
	AF083202	McKemy et al. 2005
Phialophora americana	EU514694	Untereiner et al. 2008
	EU514695	Untereiner et al. 2008
	AF050259	Untereiner & Naveau 1999
	AF050260	Untereiner & Naveau 1999
Phialophora europaea	FJ489612	Li et al. 2008
	EF551553	Zeng & Hoog 2008
	EU514698	Untereiner et al. 2008
Phialophora sessilis	AY857542	Prenafeta-Boldu et al. 2006
	AY857541	Prenafeta-Boldu et al. 2006
	DQ363414	Feldmann et al. 2006
	FJ438386	Diaz et al. 2010
Phialophora verrucosa	DQ404353	Prodi et al. 2008
	EU514701	Untereiner et al. 2008
	AF050282	Untereiner & Naveau 1999
	AF050281	Untereiner & Naveau 1999
Pyrenopeziza revincta	AJ430224	Vralstad et al. 2002
ZJ81-D5	GU981734	This paper
ZJ81-D7	GU981735	This paper
ZJ88-B3	GU981736	This paper
ZJ88-B8	GU981737	This paper

Maximum parsimony (MP) analysis was carried out using PAUP 4.0b10 (Swofford 2001). Heuristic searches were conducted with a 1000 random taxa addition and tree bisection-reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Measures calculated for parsimony included tree length, consistency index, retention index, and rescaled consistency index (TL, CI, RI and RC, respectively). To assess the robustness of clades and internal branches, a strict consensus of the most parsimonious trees was generated and a bootstrap analysis of 1000 replications was performed. The outgroup was *Pyrenopeziza revincta*.

Results

DNA phylogeny

Four isolates (ZJ81-D5, ZJ81-D7, ZJ88-B3, ZJ88-B8) were obtained from bamboo. The sequences were deposited in GenBank (ZJ81-D5 = GU981734, ZJ81-D7 = GU981735, ZJ88-B3 = GU981736, ZJ88-B8 = GU981737). Sequences for the ribosomal DNA ITS region (ITS1, 5.8S rDNA gene, ITS2) for each isolate, and related sequence data from GenBank, were used to construct a strict consensus tree with tree length (TL) = 431, consistency index (CI) = 0.8770, retention index (RI) = 0.9671, and rescaled consistency index (RC) = 0.8482 (Fig. 1). Two major clades were resolved in the MP trees. One clade, with 100% bootstrap support, contained two species, *Cadophora gregata* and *C. malorum*. The other major clade (100% bootstrap) consisted of four subclades containing isolates of *P. sessilis*, *P. europaea*, *P. americana,* and *P. verrucosa*. Our four isolates — ZJ81-D5, ZJ81-D7, ZJ88-B3, ZJ88-B8 — clustered together with *P. sessilis* with a 100% bootstrap value, indicating that they probably represent the same species.

Taxonomy

Description (Fig. 2): Hyphae initially somewhat torulose, later regularly tubular. Expanding hyphae 1.2–2.7 µm wide, smooth-walled; hyphal cells sometimes inflated to 3.8–7.5 µm wide. Phialides mostly intercalary. Collarettes distinct from the rest of the phialide, mostly sessile on undifferentiated hyphae, scattered and independent from placement of septa, triangular to funnel-shaped, up to 1.5 µm long and about 1.5 µm wide at the often somewhat flaring opening. Conidia subhyaline, smooth-walled, obovoidal to ellipsoidal, 3.0–7.3 × 2.0–4.5 µm. Conidia in slimy heads, 5.3–8.2 × 6.5–8.4 µm. Chlamydospores absent.

Specimens examined: On *Phyllostachys meyeri* McClure (*Gramineae*): China, Hubei, Xianning, Qianshan National Forest Park, 29°48'N 114°96'E, alt. 46 m, 16 Oct. 2008, J.L. Zhuang & H.L. Yang, HMUABO 20581 (with dried culture), culture ZJ81-D5 and ZJ81-

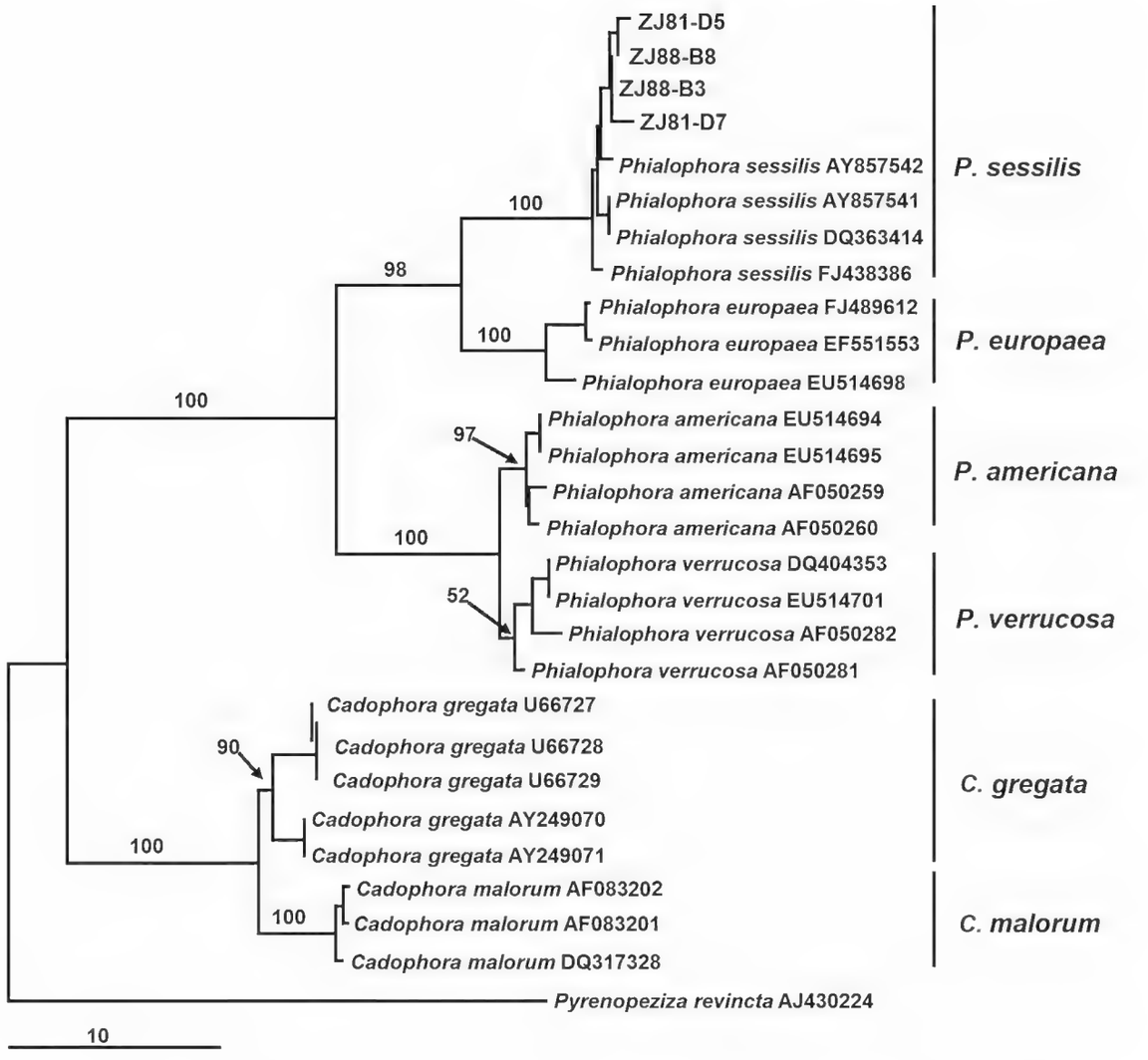

FIG. 1 The parsimony tree (TL = 431, CI = 0.8770, RI = 0.9671, RC = 0.8482) derived from a heuristic search option in PAUP version 4.0b10 with 1000 randomizations of sequence input orders and 1000 bootstrap replications using the data set of ITS1, 5.8S and ITS2. Bootstrap values higher than 50% are indicated above or below the tree branches. The tree was rooted to *Pyrenopeziza revincta*.

D7; on *Yushania falcatiaurita* Hsueh & T.P. Yi (*Gramineae*): China, Hubei, Xianning, Qianshan National Forest Park, 29°48'N 114°96'E, alt. 53 m, 16 Oct. 2008, J.L. Zhuang & H.L. Yang, HMUABO 20588 (with dried culture), culture ZJ88-B3 and ZJ88-B8.

CULTURAL CHARACTERISTICS: Colony diameter after 1 month on PDA at 22 ± 1°C reached 20 mm with even margins and rough, farinose aerial hyphae; colony centers were purplish gray and outer zones olivaceous black. On OA similar, colony reaching 23 mm diameter, flat, spreading, with sparse aerial mycelium, surface olivaceous black, but colony color lighter.

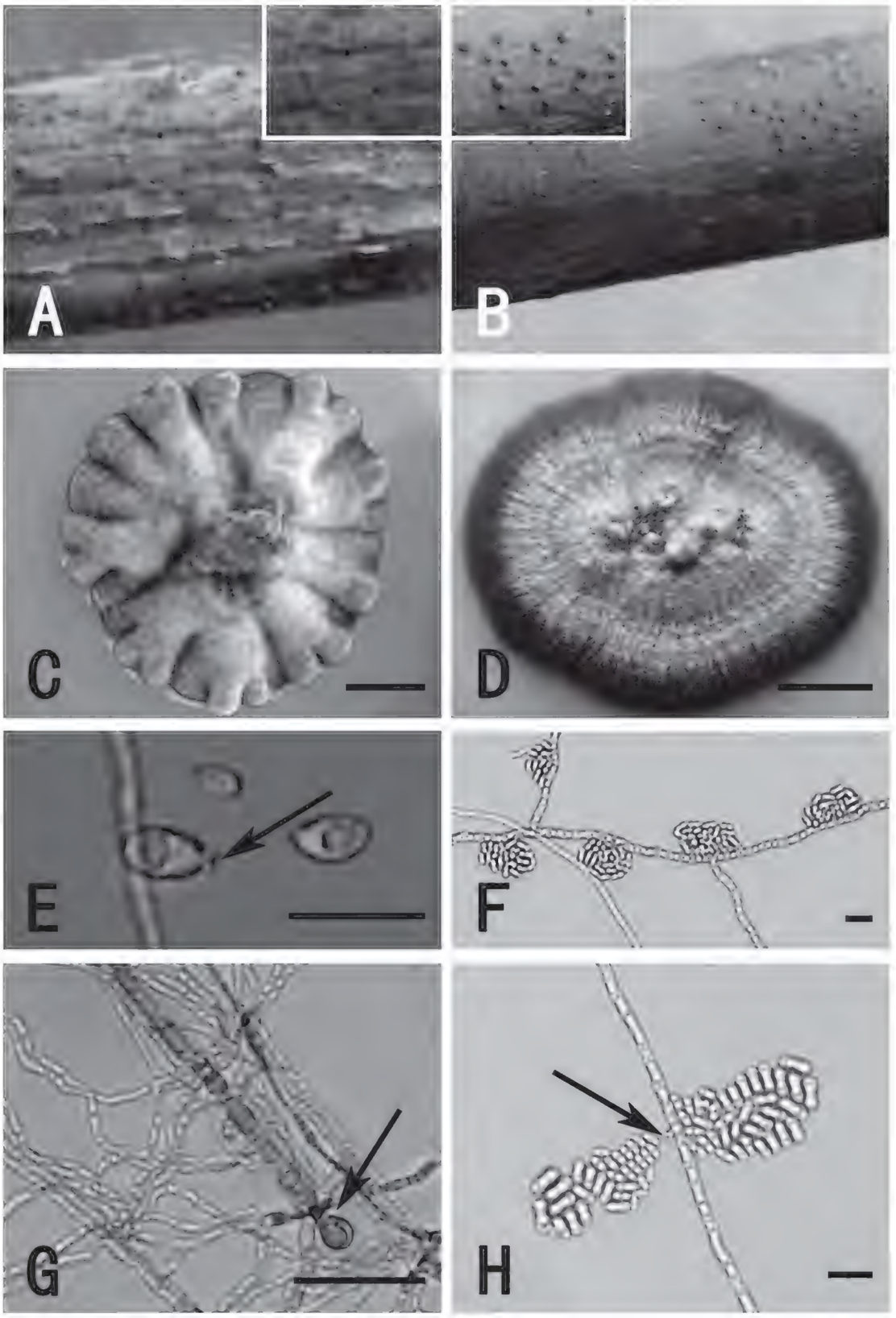

Fig. 2 *Phialophora sessilis* (ZJ81-D5). A. Signs on *Yushania falcatiaurita* with close-up view. B. Signs on *Phyllostachys meyeri* with close-up view. C. Colony on PDA after 30 days. D. Colony on OA after 30 days. E. Conidia with open collarette (arrow). F. Conidia and hyphae. G. Inflated hyphal cells (arrow). H. Phialide (arrow) bearing conidia. Bars (C–D) = 0.5 cm. Bars (E–H) = 10 μm.

Host characteristics: On *Yushania falcatiaurita*, no visible mycelial mat with shiny, black, flattened sclerotium-like bodies, round to irregular (130–470 μm diam), scattered distribution over the entire surface of the culm, densely arranged 3–8/mm^2 (Fig. 2A). On *Phyllostachys meyeri* similar, but sclerotium-like bodies were larger (430–680 μm diam), sparse, gathered in clusters on the culm, densely arranged 0.5–1.5/mm^2 (Fig. 2B). The flyspeck on bamboo did not damage the plants, but greatly reduced their ornamental and retail value. As a result, these fungi can cause significant economic losses to producers of these plants.

Discussion

Our four isolates are morphologically similar to *Phialophora sessilis* de Hoog, and despite minor differences, identity with this species was well supported by the ITS data. The same fungus caused somewhat different signs on each hosts. This phenomenon occurs in other so-called flyspeck species as well, for example, *Dissoconium mali* produced colonies with flyspeck morphology on persimmon fruit (Sun et al. 2008), but colonies with sooty blotch morphology (dark mycelial matrix) on apple fruit (Zhang 2007). It is possible that host-based morphological plasticity may also occur in other fungi in the sooty blotch and flyspeck complex.

Based on the ITS sequence analysis and morphological comparison, we identified the four isolates as *Phialophora sessilis,* which represents a new record for China. This study is also the first report of *P. sessilis* from bamboo.

Acknowledgments

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